Technical Service Report

Stability of Fluorescein-Labeled Conjugates

Purpose:

To evaluate the performance of KPL fluorescein-labeled (FITC) conjugates over a six year period.

Reagents:

On the date of manufacture, representative samples of the following lots of FITC labeled Goat Anti-Mouse IgG (H+L) (HSA) were rehydrated to 1 mg/ml in reagent quality water for initial QA testing. These samples were stored frozen after that date. Other samples were stored at 4°C from the date of manufacture in their original lyophilized form. Testing was performed in December 1993.

Lot Number	Date of Mfg.
KB07-5	3/88
LG53-2	8/89
MD36-5	5/90
NH43-2	8/91
PA20-2	1/92
QE36-5	6/93

Test Parameters:

The conjugates were evaluated using fluorescent microwell immunoassay and fluorescent slide assay procedures.

The fluorescent microwell immunoassay was performed as follows:

1. Add 100 µl Mouse IgG (Cappel; Lot 38621) diluted to 10 µg/ml in PBS to all wells of a round bottomed microtiter plate. Incubate one hour at room temperature.
2. Block the plate with 300 µl/well BSA Diluent/Blocking Solution (Lot QJ02) diluted 1:10 in reagent quality water. Incubate 10 minutes.
3. Dilute each FITC labeled Goat Anti-Mouse IgG (H+L) sample to 50 µg/ml in 1X BSA Diluent.
4. Add 200 µl of each diluted conjugate sample to the appropriate wells in Row A (see Figure 1). Add 100 µl of 1X BSA Diluent/Blocking Solution to all test wells in rows B-H. Perform serial two-fold dilutions of the conjugate by transferring 100 µl from each well in Row A to the well below. Continue to make dilutions through Row H.
5. Incubate for one hour at room temperature.
6. Wash plate 5 times with Wash Solution Concentrate (Lot PB27) using an automatic Skatron plate washer.
7. Determine the O.D. for each well using the Titertek Fluoroskan II reader.

The fluorescent slide assay was performed as follows:

1. Place 5 µl of *E. coli* strain K-12, diluted 1:400 in reagent quality water, on 12 slides. Air dry and heat fix.
2. Dilute Rabbit Anti-E. coli (Dako; Lot 016) 1:500 in KPL Milk Diluent/Blocking Solution (Lot QE05), diluted 1:20 in reagent quality water. Apply 50 µl antibody to each slide, coverslip, and incubate 30 minutes in a humidity chamber.
3. Wash slides for 5 minutes in Tris-HCl.
4. Dilute Mouse Anti-Rabbit Ig (Dako; Lot 110) 1:25 in Milk Diluent. Apply 50 µl to each slide and incubate as described in Step 2.
5. Wash slides for 5 minutes in Tris-HCl.
6. Dilute each FITC labeled Goat Anti-Mouse IgG (H+L) (hsa) sample to 25 µg/ml in Milk Diluent. Apply 50 µl to each slide and incubate as described in Step 2.
7. Wash slides for 5 minutes in Tris-HCl.
8. Rinse slides in water, air dry, apply KPL FITC Mounting Media (Lot QE49), and view with fluorescent microscope.

FIGURE 1. Optical Density Values from Various Lots of Fluoroscein Labeled Antibodies When Stored Frozen or at 4°C

	Lot KB07		Lot LG53		Lot MD36		Lot NH43		Lot PA20		Lot QE36	
	A	B	A	B	A	B	A	B	A	B	A	B
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.427	1.489	1.592	1.751	1.451	1.454	1.913	1.878	1.700	1.812	1.948	1.894
B	1.167	1.281	1.458	1.513	1.243	1.259	1.674	1.724	1.584	1.611	1.757	1.678
C	0.875	1.025	1.149	1.271	1.020	1.027	1.349	1.378	1.250	1.327	1.401	1.401
D	0.622	0.721	0.811	0.936	0.722	0.746	0.974	0.956	0.853	0.891	0.977	0.973
E	0.436	0.478	0.536	0.623	0.492	0.525	0.643	0.600	0.544	0.597	0.645	0.645
F	0.327	0.313	0.379	0.407	0.365	0.368	0.419	0.406	0.379	0.392	0.429	0.414
G	0.416	0.430	0.296	0.315	0.298	0.307	0.467	0.453	0.271	0.287	0.325	0.302
H	0.288	0.281	0.234	0.263	0.245	0.248	0.318	0.288	0.225	0.251	0.240	0.245

A = Sample rehydrated at date of manufacture and stored frozen.
B = Sample stored at 4°C in lyophilized form; rehydrated at date of testing.

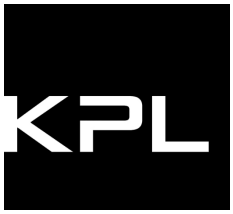
Results:

In the fluorescent microwell immunoassay, samples from the same lot that were stored under different conditions were identical in performance. When comparing the different lots, samples stored for two years or less were highest in activity. Lots that had been stored from two to six years showed slightly lower activity.

Similarly, in the fluorescent slide assay, there was no difference in performance between samples from the same lot that had been stored under different conditions, and samples that had been stored for two years or less gave the most intense fluorescence.

Conclusions:

KPL's FITC conjugates are equally stable when stored at either 4°C in lyophilized form or stored frozen after rehydration with reagent quality water. A high level of performance is seen with FITC conjugates that have been stored for up to six years. Optimal performance is obtained when FITC conjugates are used within two years of the date of manufacture.



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